EQUILIBRIUM ORGAN: STRUCTURE AND FUNCTION IN THE BLUE CRAB, **Callinectes sapidus**

By

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EQUILIBRIUM ORGAN: STRUCTURE AND FUNCTION IN THE BLUE CRAB, *CALLINECTES SAPIDUS*

By

David Byron Roye

March, 1970

Chairmen: Dr. Kent E. Chernetski (deceased) and Dr. James H. Gregg
Major Department: Department of Zoology

This study investigates aspects of the functional role of the statocysts in *Callinectes sapidus*. Anatomical features of the statocysts and regions of the central nervous system receiving input from the statocysts were also examined. The statocysts of *Callinectes* were found to contain three separate groups of receptor elements: hook, thread and group hairs. Of these three types, only the hook and thread hairs were clearly observed to be innervated by sensory neurons. The evolutionary relationships of statocyst receptor hairs in brachyuran and macruran decapods are discussed. The fiber composition of the central synaptic field receiving input from the statocysts (the antennulary neuropile) is described.

The major portion of this study is an electrophysiological analysis of higher-order units in the central nervous system responding to rotation of the statocysts. Identifiable units were found in nerves leaving the central nervous system which responded to phasic

rotation of the statocysts with bursts of repetitive activity. The repetitive outputs of these higher-order units are explained on the basis of integrative capabilities possessed by interneurons and motoneurons of other decapods. It has been possible to link the phasic activity in nerves leaving the central nervous system with the output of the thread hair receptors in the statocysts. The destruction of the receptor neurons innervating these hairs prevents additional evoked responses.

Of considerable importance was the discovery that dorsal rotation of a single statocyst produced stereotyped movements of the thoracic appendages. The higher-order units responsible for these movements are located in the circumesophageal connectives. The movements themselves result in phasic postural changes in this crab. It is likely that phasic input from the statocysts, acting on higher-order neurons in the antennulary neuropile, continually adjusts the position(attitude) of the various appendages.

GENERAL INTRODUCTION

The role of statocysts in determining body orientation with respect to gravity has been well established, particularly in the decapod Crustacea (Kreidl, 1893; Schöne, 1951, 1954; and Dijkgraaf, 1956a, 1956b). For the most part, investigators have been concerned with the postural changes which accompany the rotation of experimental animals about their body axes, and not with the neural mechanisms which underly these changes.

Cohen (1955, 1960) and more recently Patton (1969) have studied the sensory input from the statocyst to the central nervous system, but the manner in which the central nervous system integrates this input and initiates appropriate responses is largely unknown.

Studies by Cohen and Maynard (1961) and Maynard (1967) have revealed much about the integrative capabilities of single crustacean interneurons through the electrical stimulation of specific inputs (including that from the statocyst) to the central nervous system. Again, however, few if any attempts have been made to relate these findings to normal behavior.

The primary objective of the following study has been to investigate activity in neurons in the central nervous system responsible for orienting behavior in Callinectes sapidus. It has been possible to associate activity in some of these neurons with stereotyped movements of the thoracic appendages. These movements are most obvious in the heterolateral appendages during phasic rotation of a single statocyst and are believed to initiate the return to a normal dorsal-side up posture in this species.

The first part of this study is an investigation of the anatomy of the statocysts together with the anatomy of those regions of the central nervous system receiving input from the statocysts. The second part is an electrophysiological investigation of higher-order neurons in the central nervous system responding to phasic stimulation (i.e., rotation) of the statocysts. The preparation used was well suited for this study and should be useful in other investigations of nervous integration in the Crustacea.

PART I

STRUCTURAL ORGANIZATION OF THE STATOCYSTS AND RELATED PORTIONS OF THE CENTRAL NERVOUS SYSTEM

Introduction

An investigation of the statocysts of *Carcinus maenas* by Dijkgraaf (1956b) describes two functionally important types of receptors: hook and thread hairs. In intimate contact with many of the hook hairs are minute statoliths (25μ in diameter) previously believed to be lacking in all crabs (Prentiss, 1901; Hensen, 1863; and Schöne, 1954). A portion of this study is devoted to the anatomical investigation of the statocysts of *Callinectes*, with particular emphasis on the types of receptors present. An attempt was made to determine whether or not statoliths were associated with these receptors.

Both types of receptors were found in *Callinectes*, but statoliths were not observed. In size and shape the hook and thread hairs of *Callinectes* closely resemble those found in *Carcinus*. Additionally, the thread hairs of both forms were similar to those found in *Homarus americanus*, but the hook hairs in both crabs were considerably reduced in size in comparison with the homologous statolith hairs of *Homarus*.

The central nervous systems of the decapod crustacea have been studied extensively (Hanstrom, 1929; Helm, 1928; and Bullock and Horridge, 1966). Up to now, the central nervous system of *Callinectes* has not been investigated, but those of similar brachyuran crabs, including *Carcinus*, have been studied (Bethe, 1897, and Hanstrom, 1929). In both

Callinectes and Carcinus, the major component of the central nervous system, the cerebral ganglion, is essentially the same. In this study of Callinectes, close attention was paid only to regions of the cerebral ganglion in which components of the antennulary nerve terminated.

Particular attention was given to the antennulary neuropile, since this synaptic field appeared to receive most of the primary sensory input from the statocyst. The antennulary neuropile of Callinectes was found to be similar to the comparable neuropile in decapods generally, being a diffuse, tangled network of branching axons with no apparent anatomical organization. Elements sending axonal processes into this neuropile were bipolar first-order neurons with cell bodies located in the statocyst and higher-order neurons with cell bodies located just outside the neuropile itself.

Activity in higher-order neurons leaving the antennulary neuropile and cerebral ganglion through the various efferent ganglionic nerves is considered in Part II of this study.

Methods

All crabs used in this study were caught by commercial crabbers off the gulf coast of Florida near Cedar Key. Within hours of their capture, healthy specimens were returned by car to Gainesville, Florida. During the 45-minute trip from Cedar Key to Gainesville, crabs were kept cool in an aluminum chest containing a bag of ice. In Gainesville they were immediately placed in a 20-gallon tank of artificial sea water (commercially prepared by the Ruet Company, Brooklyn, New York) maintained at 22°C. Further cooling was not necessary. In addition to a large undergravel filtering and aerating system, two Metaframe Dynaflo outside filters were employed to insure against fouling of the water by the crabs and to increase the carrying capacity of the tank. Under these conditions roughly two dozen crabs could be maintained for periods up to six weeks. Except for an initial stressful period lasting for a few days after capture, crabs remained extremely healthy and active until used.

The statocysts of Callinectes are contained within the basal segments of the antennules. These segments, and their innervation, were studied with the aid of a dissection microscope. Fine anatomical features of the segments were photographed with the aid of a Wild-Heerbrugg compound microscope. Fresh preparations of statocyst hairs were photographed through a Zeiss compound microscope.

Also prepared were horizontal and transverse serial sections of isolated basal segments. These segments are attached to the posterior wall of an exoskeletal cup which surrounds them on all but the anterior side. Segments were removed by cutting this attachment and the antennulary nerve.

After their removal, segments were placed immediately in 10% formalin/distilled water (3 days), followed by a solution of trichloracetic acid (7-10 days). They were then washed for 24 hours in running water and placed for one-week periods in 2, 4, 6 and 12% celloidon. Following this they were imbedded in 12% celloidon and cut with an American Optical Company (Model 860) microtome at 25 and

40 micra. Sections were numbered and stored in 70% ethyl alcohol until time for staining. Receptor neurons in the basal segments were stained using the method of Kluver and Barrera (Conn _et al_., 1962).

The following procedures were used in preparing cerebral ganglia for horizontal serial sectioning: At the outset, chelae and mouthparts were removed from healthy specimens and the body cavity exposed ventrally in the region of the esophagus. Connective tissue around each ganglion was peeled away and the area perfused with 10% formalin. Nerves entering the ganglia were cut a few millimeters from their point of entrance. Ganglia were then lifted free and stored in 10% formalin. Prior to paraffin embedding ganglia were washed in running water (8 hours), dehydrated in 2-ethoxyethanol (4 hours) and cleared in cedarwood oil overnight. They were then transferred to equal parts cedarwood oil, benzene and paraffin (37°C for 1-1½ hours). Ganglia were then imbedded in paraffin (56°C), sectioned at 15 micra and placed on albuminized slides.

Two staining procedures were used to stain nervous elements in the cerebral ganglia. Thionin (Clark and Sperry, 1945) was used to stain Nissl substance within cells and the Guillery _et al_. modification of the Nauta-Gygax technique (1962) was used to stain normal fibers.

Methods used to stain nervous elements in the statocysts and cerebral ganglia are summarized below.

Kluver and Barrera Method:

1. 80% ethyl alcohol (2-3 minutes).
2. 95% ethyl alcohol (2-3 minutes).

3. Staining solution: Luxol fast blue, 1 gm; 95% ethyl alcohol, 1000 mls; 10% acetic acid, 5 mls (24 hours).
4. 95% ethyl alcohol (2-3 minute rinse).
5. Wash in distilled water (5 minutes).
6. Differentiation of individual sections in 0.05% aqueous lithium carbonate solution for a few seconds.
7. 70% ethyl alcohol (several changes, a few seconds in each).
8. Wash in distilled water (5 minutes).
9. Dehydrated in a series of alcohols to 95%.
10. Clear in terpineol, terpineol-xylene, xylene and mount.

Thionin Method:

1. Paraffin removed in xylene.
2. Hydration to distilled water.
3. 0.55% lithium carbonate solution (5 minutes).
4. 0.25% thionin in 0.5% lithium carbonate (5-10 minutes).
5. Wash in distilled water (2-3 minutes).
6. 70% ethyl alcohol (seconds).
7. Dehydrate in 2 changes of butyl alcohol (3 hours each), clear in xylene, and mount.

Guillery Method:

1. Paraffin removed in xylene.
2. Hydration to distilled water.
3. 1% ammonium hydroxide (specific gravity 0.09) in 50% ethyl alcohol (6 hours).

4. Wash in three changes of distilled water.
5. Ammoniacal silver solution: 1/5% silver nitrate, 95 parts; white pyridine, 5 parts (left in dark for 24 hours).
6. 4.5% silver nitrate, 20 mls; absolute alcohol, 10 mls; ammonium hydroxide (specific gravity 0.09), 2.0 mls; 2.5% sodium hydroxide, 1.5 mls. (This solution was placed on a hot plate with slides in it and removed when the temperature reached 40-45°C. No more than three slides were taken through at one time and the solution was changed frequently.)
7. Reducing solution: 10% ethyl alcohol, 450 mls; equal parts 1% citric acid and 10% formalin, 35 mls (1-2 minutes).
8. Wash in distilled water.
9. 1% sodium thiosulfate (1 minute).
10. Wash in distilled water, dehydrate in ethanol, clear in xylene and mount.

Results

Structure of the Basal Segment

The statocysts of Callinectes are encased within the enlarged basal segments of the antennules. Each basal segment is oval in shape and dorsally flattened. The proximal segment of the antennule projects antero-dorsally from the medial aspect of the segment, with the musculature responsible for its movement about the basal antennular joint occupying the medial one-third of the interior of the segment. The dorsal surface of the basal segment is split into anatomically distinct anterior and posterior sections. It appears that during the course of development of the basal segment, the anterior portion overlaps the posterior portion dorsally, with the anterior section eventually becoming considerably thicker and more heavily pigmented. Both dorsal and ventral surfaces of the basal segment are covered with numerous tactile hairs (Figure 1).

Each basal antennular segment is enclosed within an exoskeletal depression at the anterior end of the animal medial to the antennae. The basal segment is not immobilized by the exoskeleton and there exists a pair of antagonistic muscles (the promotor and remotor muscles of the first antennae) which attach the posterior portion of the segment to the exoskeleton (Cochran, 1935). These muscles are capable of rotating the segment in its socket. The promotor muscle is located dorso-medially and acts to raise the basal segment and rotate it slightly towards the midline. This muscle does not insert on the exoskeletal portion of the posterior wall of the segment, but rather passes through an opening and inserts on the thickened posterior wall of the statocyst sac itself.

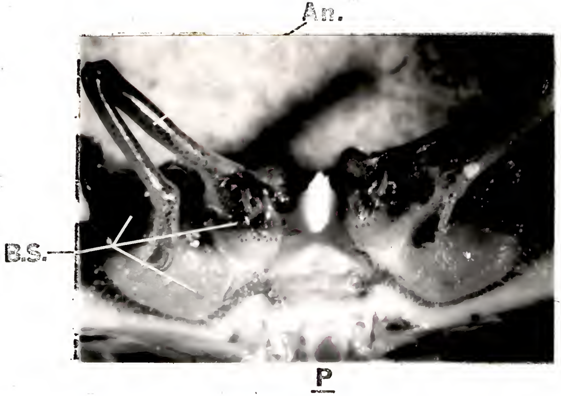
The remotor muscle is found ventro-lateral to the promotor and acts by pulling the basal segment downward and rotating it somewhat laterally in its socket. In contrast to the promotor muscle, the remotor inserts on the outer exoskeletal wall of the basal segment. Located immediately dorsal to the promotor muscle is a joint-like articulation of the exoskeletal components of the basal segment and body wall. An elongate portion of the body wall fits into a socket-like extension of the postero-lateral corner of the basal segment. The effect of this "joint" is to anchor the postero-lateral corner of the segment and in so doing produce the rotation and slight medio-lateral roll associated with the actions of the antagonistic muscles.

Structure of the Statocyst

In *Callinectes* the statocyst is a ventrally directed invagination of the dorsal wall of the basal antennular segment (Figure 2). Due to a considerable number of bizarre indentations, the statocyst is rather difficult to describe, but basically it is cone-shaped with a dorsally elongate base and ventrally projecting apex. The two ends of the elongate base of the cone are directed antero-laterally and postero-medially. The statocyst is a fluid-filled sac completely closed to the outside in the adult crab. Prentiss (1901) has demonstrated in *Carcinus* that a slit-like opening is present during molting. The thickness of the sac wall varies, with the anterior surface being quite thin and easily pierced with a dissecting needle. The posterior wall is reasonably thick and serves as a surface for the insertion of the promotor muscle. As mentioned, the outer surface of the sac is highly convoluted with the largest and deepest invagination occurring on the antero-medial wall of the sac.

Figure 1. Dorsal view of the basal segments and antennulae. Anterior portion of the carapace removed. An.- Antennule; B.S.- basal segment; P - posterior. Magnification: 4.2x.

Figure 2. Ventral view of the left statocyst following removal of the bottom half of the left basal segment. L - lateral; P - posterior; S.- statocyst. Magnification: 9.6x.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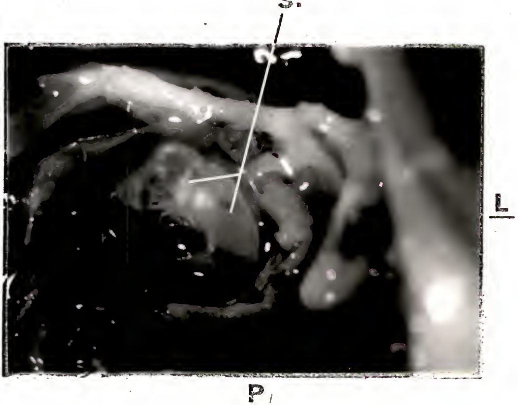
S.
L
P/

Projecting into the interior of the statocyst are groups of sensory hairs (sensory cushions). Three types of receptor hairs have been described in a thorough cross section of the Brachyura (Hensen, 1863, and Prentiss, 1901) and are here confirmed to be present in Callinectes. Each type is restricted to a specific area of the inner surface of the sac. The first type, called hook hairs (Figures 3, 4 and 5) because most possess hooked shafts, are located on the posterior wall of the sac on a slight protuberance. These receptors were grouped together in no obvious pattern. Fine bristles were observed projecting out from the more distal regions of these hairs. Hook hairs were short, having a mean length of 158μ. The diameter of these hairs averaged 14μ.

A second group of sensory hairs, the thread hairs, was found on the inner surface of the antero-medial invagination of the sac (Figures 3, 6 and 7). In contrast to the hook hairs, these receptor hairs occurred in a single row. Thread hairs were long (roughly 750μ) and slender (14μ) and possessed tufts of bristles at the tip.

The last receptors found have been termed group hairs (Hensen, 1863). These hairs were observed as an irregular mass of rather large hairs located in the antero-lateral corner of the sac. Group hairs were the same length as thread hairs but considerably thicker, averaging 47μ in diameter (Figures 3, 8 and 9). In appearance they more closely resembled tactile hairs than did either of the other two receptor types. Statoliths were not found in association with any receptor hairs in

Figure 3. Dorsal view of a horizontal section through the right basal segment. Illustration depicts the relative positions of the three groups of receptor hairs. A - anterior; An.M.- musculature of the antennule; C.- central component of the antennulary nerve; G.- group hairs; H.- hook hairs; L.- lumen of the statocyst; M - medial; R.N.-receptor neurons; T.- tactile hairs; Th.- thread hairs.

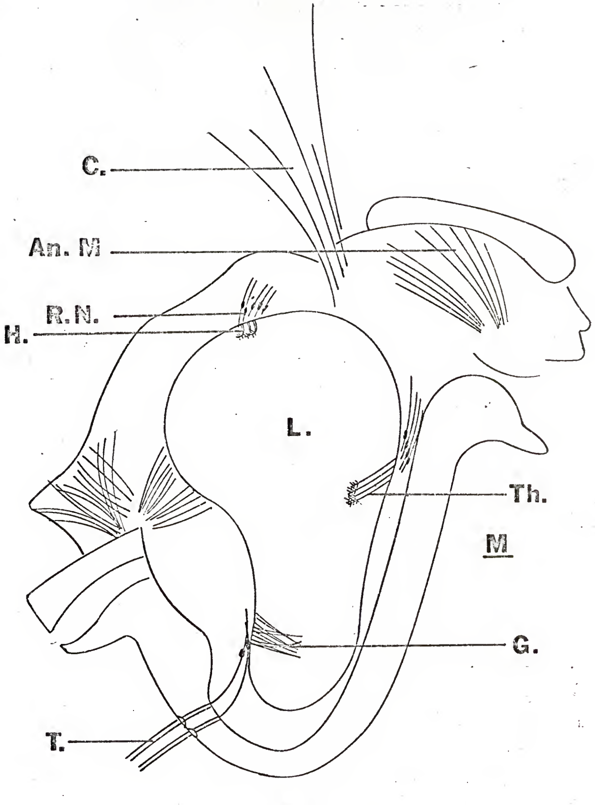
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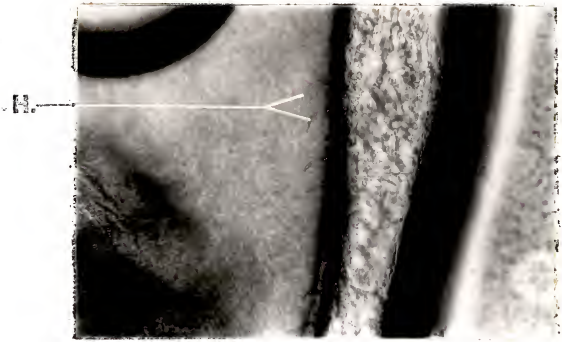
Figure 4. Phase contrast photomicrograph of a number of hook hairs. H.- hook hairs. Magnification: 100x.

Figure 5. Illustration showing the positions of hook hair receptors in Figure 4.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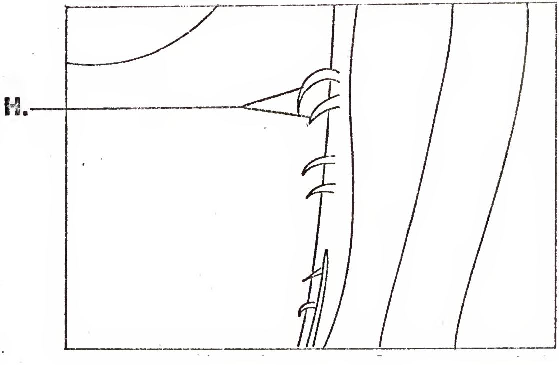
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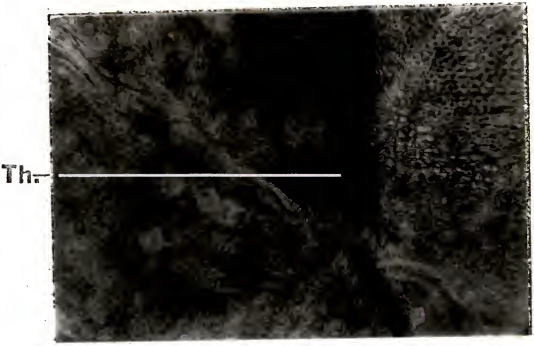
Figure 6. Phase contrast photomicrograph of the thread hair row. Th.- thread hair. Magnification: 100x.

Figure 7. Illustration showing the positions of thread hair receptors in Figure 6.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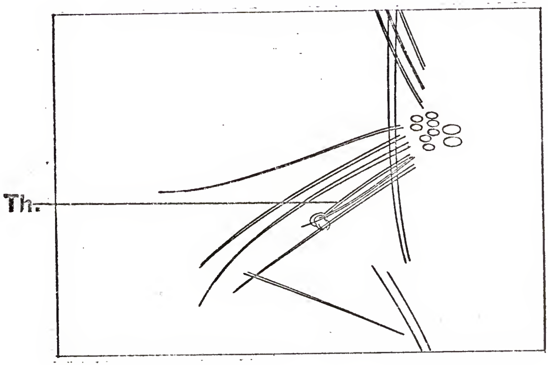
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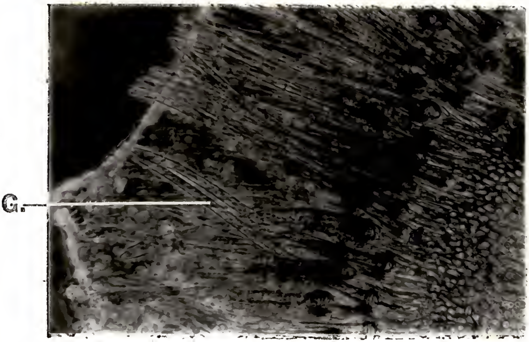
Figure 8. Phase contrast photomicrograph of the group hair sensory cushion. G.- group hairs. Magnification: 100x.

Figure 9. Illustration showing the positions of the group hairs in Figure 8.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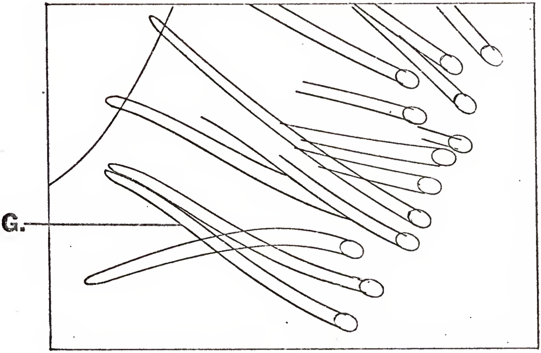
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fresh material, even after magnification of the sensory cushions containing these receptors 50 diameters.

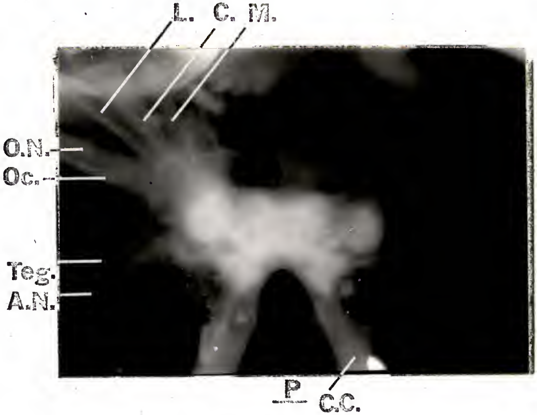
Innervation of the Basal Segment

The aggregation of nerve bundles which exits ventrally from the antero-lateral corner of the cerebral ganglion and passes into the basal segment of the antennule is generally referred to as the antennulary nerve (Figures 10 and 11). In addition to fibers passing to the antennule, it contains bundles from the statocyst (receptor hairs) and basal segment (tactile hairs and muscles) as well. In Callinectes, the basal segment is quite close to the cerebral ganglion and consequently all bundles are quite short, the distance from the ganglion to the basal segment being only a few millimeters. Two medial bundles pass through an opening in the posterior wall of the basal segment in association with the others, but once inside pass dorso-medially into the antennule after supplying antennulary muscles in the basal segment itself. Centrally located bundles project to the apex of the statocyst sac, from there spreading out dorsally to innervate the hook and thread hairs. A single lateral bundle innervates the muscles and tactile hairs of the basal segment.

Neurons innervating hair receptors in the statocyst were revealed in serial sections of the basal segments (Figure 12). Numerous bipolar neurons were seen in the connective tissue matrix outside the sac and beneath the fields of receptor hairs. Clusters of cells were observed beneath hook and thread hairs in particular. The somata of these

Figure 10. Ventral view of the cerebral ganglion and associated nerves. A.N.- antennal nerve; C.- central component of the antennulary nerve; C.C.- circumesophageal connective; L.- lateral component of the antennulary nerve; M.- medial component of the antennulary nerve; Oc.- oculomotor nerve; O.N.- optic nerve; O.Neu.- olfactory neuropile; P - posterior; S.- ganglionic sheath. Magnification: 9.6x.

Figure 11. Illustration showing the relative positions of the ganglionic nerves indicated in Figure 10.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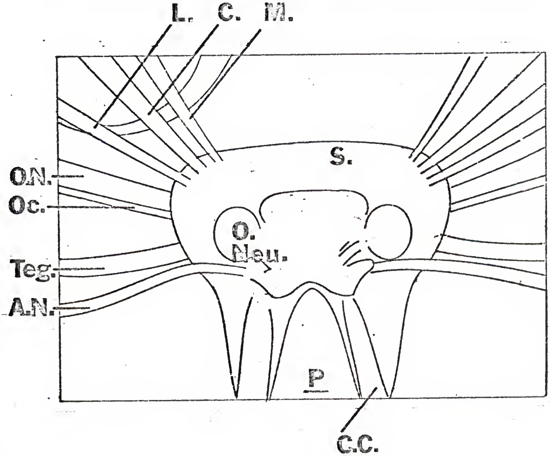
L.
C.
M.
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receptor neurons had an average diameter of 16μ. The distal processes of these cells projected to the wall of the sac and, in the case of the thread hairs, were observed passing into the hairs themselves.

Central Ramifications of the Antennulary Nerve

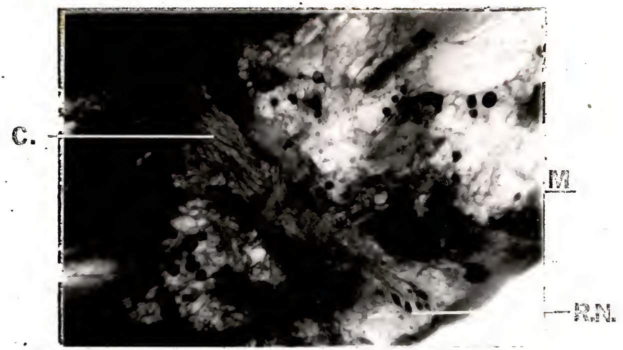
The central projection areas of the antennulary nerve were revealed in fresh unstained preparations of the cerebral ganglion examined under the dissection microscope and in horizontal serial sections of cerebral ganglia impregnated with silver (Figure 13). Inside the ganglion the medial bundles were observed passing dorsal to fibers emanating from the central and lateral bundles. Fibers from the medial bundles were believed to terminate in the neuropile of the olfactory lobe. The rest of the antennulary nerve continued medially over the ventral surface of the ganglion and terminated in a diffuse neuropile (the antennulary neuropile) postero-medial to the olfactory lobe (Figures 10, 11 and 13).

Gross Anatomy of the Central Nervous System of Callinectes

All major nerves associated with the cerebral ganglion are shown in Figures 10 and 11. In addition to these nerves, two long circum-esophageal connectives join the cerebral ganglion with the thoracic and abdominal ganglia. In *Callinectes* these paired, segmental ganglia are fused into a ring. Examination under a dissection microscope revealed the abdominal ganglia to be much reduced in size (as are the abdominal segments in these crabs) in comparison with the five pairs of

Figure 12. Dorsal view of a horizontal section through one of the basal segments showing a cluster of bipolar receptor neurons at the base of the thread hair sensory cushion. A - anterior; C.- central component of the antennulary nerve; M - medial; R.N.- receptor neuron. Magnification: 60x.

Figure 13. Ventral view of a horizontal section through the cerebral ganglion. Labeled are the right antennulary and olfactory neuropiles (compare with Figure 10). An. Neu.- antennulary neuropile; C.- central component of the antennulary nerve; O.Neu.- olfactory neuropile; P - posterior. Magnification: 60x.



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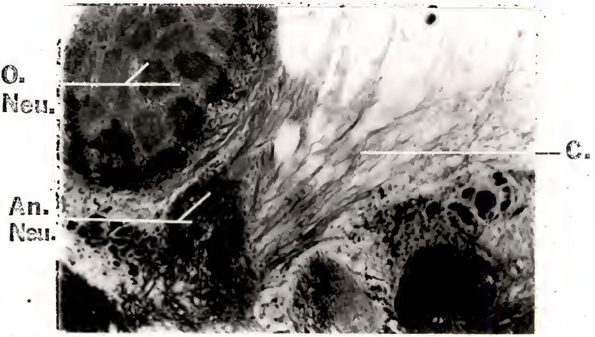

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thoracic ganglia. The five pairs of thoracic ganglia receive large nerves from the thoracic appendages.

Discussion

Comments on the Anatomy of Statocysts in Decapods

The structural organization of the decapod statocyst has been the subject of numerous comprehensive studies (Hensen, 1863, and Prentiss, 1901). In the Macrura (e.g., Homarus) statocysts are open to the outside and contain large statoliths in contact with statolith hairs approaching 850μ in length. Hairs not in contact with the statolith reach a length of 750μ and are thinner than the statolith hairs. The fragile appearance of these receptor hairs has resulted in their being termed thread hairs.

Callinectes has been shown to possess closed statocysts containing three morphologically distinct types of receptor hairs (the hook, thread and group hairs). Of these three types only the hook and thread hairs are known to be functionally important and are the only ones which clearly receive dendritic processes. The thread hairs of Callinectes are similar to those of Homarus, being nearly 750μ long and quite thin. Hook hairs, with an average length of 158μ, bear little resemblance to either receptor type found in Homarus. The hook and thread hair receptors of Carcinus are essentially the same as those found in Callinectes.

Dijkgraaf (1956b) has reported finding minute statoliths (25μ in diameter) in contact with hook hair receptors in Carcinus. Prior to this no one reported their presence in the Brachyura, despite the considerable number of species investigated. Statoliths were not found in association with hook hairs in Callinectes. If present in this form, statoliths must be smaller than 15μ in diameter.

Dijkgraaf's findings support the contention that brachyuran hook hairs and macruran statolith hairs are homologous despite the considerable difference in their length. Prentiss (1901) alludes to the somewhat degenerate state of the hook hairs in Carcinus in his comparison of the lengths of hook and thread hairs in larval and adult forms. Hook hairs remain constant in size from larvae to adult while thread hairs increase three and one-half times in size. Furthermore, the basal segment in the adult is roughly ten times as large as that of the larval stage.

Clearly, the hook hairs of Callinectes and other Brachyura are considerably smaller than the statolith hairs of macruran forms. Nevertheless, statoliths have been found in contact with both of these receptor types, and it seems likely that both decapod groups evolved from an ancestral form which possessed statoliths in association with statolith hairs. The failure of hook hairs in Carcinus to increase in size from larvae to adult during a time when thread hairs do increase in size suggests that the structure of brachyuran hook hairs is derived rather than primitive.

The morphological differences described above might indicate functional differences between hook and statolith hairs, but proof of this will have to await more critical investigations of the function of both these receptor types. It is clear from the work of Kreidl (1893), Cohen (1955) and Dijkgraaf (1956b) that both hook and statolith hairs are concerned with positional (gravitational) reflexes. In addition, Dijkgraaf (1956b) has shown that cutting fiber bundles which innervate the thread hairs and hook hairs not in contact with any of the statoliths (free hook hairs) results in the loss of rotational reflexes in Carcinus.

Innervation of the Statocysts in Callinectes and Carcinus

The innervation of the statocyst in Callinectes parallels that found in Carcinus (Prentiss, 1901). In both species nerve bundles making up the central component of the antennulary nerve terminate at the base of the hook and thread hair sensory cushions. In Callinectes both of these receptor types are innervated by elongate bipolar neurons whose cell bodies average 16μ in diameter.

Prentiss (1901) was unable to find innervated group hairs in Carcinus. This study confirms Prentiss's findings with respect to Callinectes. A few large somata were found near the base of the group hair sensory cushion, but these cells sent processes into tactile hairs on the outer surface of the basal segment and not into the group hairs (Figure 3). Dijkgraaf (1956b) has been able to explain positional and rotational reflexes in Carcinus on the basis of hook and thread hair function alone, although Prentiss (1901) has shown that group hairs are present in this species.

In Callinectes group hairs reach a length of 750μ and a width of 47μ, making them the largest of the three types of statocyst hairs. In general appearance they resemble tactile hairs more than either of the other receptor types found in the statocysts. However, they differ from tactile receptors and the other statocyst hairs as well in that they do not possess tufts of bristles projecting outward from the distal end of the hair shaft. In serial sections, cellular material can be seen entering the hollow shafts of these hairs from outside the sac. It is therefore possible that these hairs are innervated. However, the failure of this investigator to find any cell bodies inside the basal segment other than those going to hook, thread and tactile hairs suggests that these hairs are not innervated. More research will be necessary to determine whether or not group hairs have a role in statocyst function.

Comments on the Anatomy of the Decapod Central Nervous System

As stated previously, centrally directed axons from the bipolar sense cells in the statocyst pass over much of the cerebral ganglion before terminating in a diffuse neuropile in the deutocerebrum, the antennulary neuropile (Bethe, 1897, and Helm, 1928). Maynard (1962) characterizes the diffuse neuropile of decapods as a "tangled confusion" of nerve cell processes which are uniformly tortuous, extensively branched and widely variant in diameter, i.e., "unstructured."

Neurons contributing to this type of neuropile include monopolar elements of average or large size (interneurons and motoneurons) and peripheral bipolar sense cells whose axonal endings ramify centrally. Despite the seeming lack of clear anatomical organization in regions of diffuse neuropile, individual neurons appear to have specific domains of arborization (Maynard, 1962). Maynard (1967) and Cohen and Maynard (1961), as a result of studies involving the macruran species *Homarus* and *Panulirus argus*, have separated the neuropile of the cerebral ganglion into three functional divisions.

One of these divisions (Division II) is composed of the central neuropile of the proto-, deuto-and tritocerebra and includes the antennulary neuropile. Maynard (1967) states that this division " . . . is probably largely concerned with coordinating total organism activity and maintaining orientation within the immediate environment." He suggests that elements in each of the three major functional divisions are intrinsically capable of quite different integrative processes. Interneuronal elements in Division II have wide-spreading dendritic arborizations and appear to communicate intracellularly with propagated spikes. These units are capable of intracellular occlusion so that, in the presence of input from one locus, all other inputs may be virtually ineffective (Maynard, 1967).

The relevance of these findings with regard to this study will be made clear in a subsequent discussion of the properties of higher-order neurons found in the cerebral ganglion of *Callinectes*.

PART II

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF UNITS IN THE CENTRAL NERVOUS SYSTEM RESPONDING TO PHASIC ROTATION OF THE STATOCYSTS

Introduction

A brachyuran decapod (Callinectes) was selected for use in the present study for two reasons: its ready availability and the location of the statocysts. Statocysts in macruran decapods (e.g., Homarus) are immobilized at the base of the antennules. The statocysts of most crabs, including Callinectes, are located externally and are freely movable. As a consequence it was not necessary in this study to rotate whole animals as was done in earlier investigations. Crabs could be rigidly held in one position and the statocysts alone rotated, thereby simplifying the recording of nervous activity. Even though rotation of the statocysts about the transverse axis was limited to an arc of three degrees, this was sufficient to permit adequate stimulation.

By rotating either of the statocysts independently, it was possible to evoke activity in neurons originating in the central nervous system, as well as in first-order neurons from the statocysts. One aim of this research was a study of the responses of these higher-order neurons. This study would not have been practical in a macruran type of decapod because of the need to rotate whole animals. Such rotation would make electrophysiological recording difficult if not impossible. Cohen (1956) and Patton (1969) were able to avoid this problem to some degree in Homarus by manipulating statocyst hairs directly. In doing so they were able to record efferent activity in the oculomotor nerves in response to bending of the receptor hairs. Bush et al. (1964) and Wiersma and Mill (1965) in their studies of Podophthalmus vigil and Procambarus clarkii describe units in the optic nerves and circumsophageal connectives which are sensitive to phasic stimulation of homolateral and heterolateral statocysts. They found no units which exhibited any form of sustained response to tonic stimulation.

In this investigation of the properties of higher-order units sensitive to rotation of the statocysts, particular attention was paid to the number of responding units in a given nerve and their frequencies of response. The properties of repetitive higher-order units identified in many preparations were similarly analyzed. The form of the repetitive activity in the higher-order units is the same as that evoked in higher-order units by electrical stimulation of antennulary nerve bundles (Cohen and Maynard, 1961).

Also described are stereotyped movements of the thoracic appendages accompanying dorsal rotation of a statocyst in crabs clamped ventral-side up. It is felt that dorsal rotation of one statocyst signals a side-over-side rotation of the whole animal, with movements resulting from rotation effecting a rapid return to the normal dorsal-side up posture of these crabs. "Sculling" movements of the heterolateral fifth pereiopods were implicated in the postulated orientation behavior.

Methods

Spike activity was recorded from the antennulary neuropile and efferent ganglionic nerves following exposure of the cerebral ganglion ventrally. This was accomplished by removing the chelae, portions of the exoskeleton and mouthparts with the aid of a dissection microscope. A ventral approach to the ganglion spared the major vessels to the brain and preparations lasted for as long as one and one-half hours. The slow loss of blood from the exposed haemocoel was believed to be the primary cause for the eventual "running down" of the ganglion.

A ventral approach was also used in experiments requiring the exposure of nerves passing to the fourth and fifth thoracic appendages. When recording from distal portions of a nerve, only the exoskeleton and musculature in the appendage itself were removed. Recording from proximal portions of a nerve necessitated the removal of sections of the ventral body wall and underlying musculature. Both approaches resulted in considerable loss of blood and preparations seldom lasted longer than one-half hour.

The basal segments of the antennules containing the statocysts were exposed both dorsally and ventrally by cutting away the surrounding exoskeleton. With the crabs held ventral-side up in a U-shaped clamp, a micromanipulator with a needle probe was used to rotate either statocyst through a fixed three-degree arc in the vertical plane. Rotation of the statocysts in the horizontal plane was not possible. The duration of a rotational stimulus (whether dorsal or ventral) was held constant at 100 milliseconds (± 10 milliseconds).

With the experimental animal prepared as described above and clamped inside a Faraday cage, nerves were sectioned distally and the portion of the nerve still attached to the ganglion lifted from the body fluid by means of a fine platinum wire electrode. When recording from the antennulary neuropile, a Cerrolow-filled micropipette (tip diameters: 1-10μ) was used as the active electrode. In both instances a reference electrode was placed nearby in moist tissue. Spike potentials were amplified by a Grass Instrument Company transistorized P-15 AC preamplifier connected in series with a Grass P-9B amplifier and displayed on one beam of a Tektronix dual beam oscilloscope (Model 502). A Grass Instrument Company audio-monitor-speaker system (Model RPS 109/Am4 A) was employed to aid in the identification of individual units. Activity was most often represented as a standing spot on the oscilloscope screen and photographed with a Grass Instrument Company Kymograph camera (Model C4J) and Kodak Linagraph paper film Kind 1732.

The direction and extent of rotation was monitored by means of a potentiometer connected to a dry cell, the output from which was displayed on the second beam of the dual beam oscilloscope. The casing of the potentiometer was fastened to the knob of the micromanipulator producing the rotation. By holding the shaft of the potentiometer in a fixed position and turning the manipulator knob, rotation of the statocyst in the vertical plane was translated into a vertical deflection of the oscilloscope beam.

A Grass S-4 stimulator was used in experiments concerned with stimulation of the central component of the antennulary nerve and antidromic stimulation of the homolateral optic nerve and circumesophageal connective. All nerves were exposed using the ventral approach previously described. Both the optic nerve and circumesophageal connective were cut several centimeters from the cerebral ganglion. Portions of the nerves still attached to the ganglion were placed on bipolar hook electrodes. The central component of the antennulary nerve was stimulated between a saline-filled pipette (tip diameter 0.5 millimeters) and an indifferent platimun electrode. When recording from the central component, bundles were cut distally and lifted from the body fluid on a single hook electrode. Again a reference electrode was placed in moist tissue near the cerebral ganglion.

Results

Activity in First-Order Afferent Units in the Antennulary Nerve

Despite the short length of the antennulary nerve, it was possible to record from medial, lateral and central components. Each component was cut proximal to its point of entrance into the cerebral ganglion, placed on a single hook electrode and lifted above the body fluid. The medial component of the antennulary nerve did not contain units sensitive to rotation of the statocyst, but did contain elements responding to tactile stimulation of the antennule along its entire length. Stimulation of the distal antennulary segments, in particular, produced bursts of activity. Proprioceptive elements were inferred from activity accompanying rotation of the antennule about its proximal joint.

The lateral component of the antennulary nerve contained units responding to tactile stimulation of the sensory hairs investing the basal segment. In these units, spike amplitude was several orders of magnitude greater than in tactile units found in the medial component. Proprioceptive elements associated with the promotor and remotor muscles of the basal segment were not investigated.

Afferent activity in the central component of the antennulary nerve was confined to units innervating the receptor hairs of the statocysts. The typical response obtained from any of the several bundles in the central component is given in Figure 14A. Though not apparent in the figure, a constant level of background activity is always found in fresh preparations. As can be seen, a considerable number of units respond (some repetitively) to activation of the statocyst receptor hairs by rotation of one statocyst. Other units respond repetitively when the statocyst is rotated and held in a new position. Units responding to rotation of a statocyst were quite common, but units responding to maintained positions were found infrequently.

It was possible to observe activity in small numbers of units by carefully splitting large bundles into smaller ones. Figure 14B shows the response patterns of units in such a small bundle. The active portion of the bundle is composed primarily of units responding to statocyst rotation, but several units can be seen which fire repetitively in response to maintained position. The rotational units in this figure can be divided into two classes: units activated by rotation of the statocyst in one direction, and units activated by rotation of the statocyst in the other. Units responding to rotation in one direction usually did not respond to rotation in the opposite direction.

In Figure 14C a small bundle containing a single active positional unit is shown. The unit fires repetitively yet has no consistent interspike or interburst interval. The number of spikes in a burst varies. This bundle also contains several small amplitude rotational units.

Activity in Higher-Order Units Originating in the Central Nervous System

Rotation of a single statocyst dorsally was frequently associated with stereotyped movements of the pereiopods. These movements were most obvious in the fifth pereiopods, particularly the heterolateral one. In view of these movements, the responses of descending units in the circumesophageal connectives and efferent units in the thoracic leg nerves during rotation of a single statocyst were investigated.

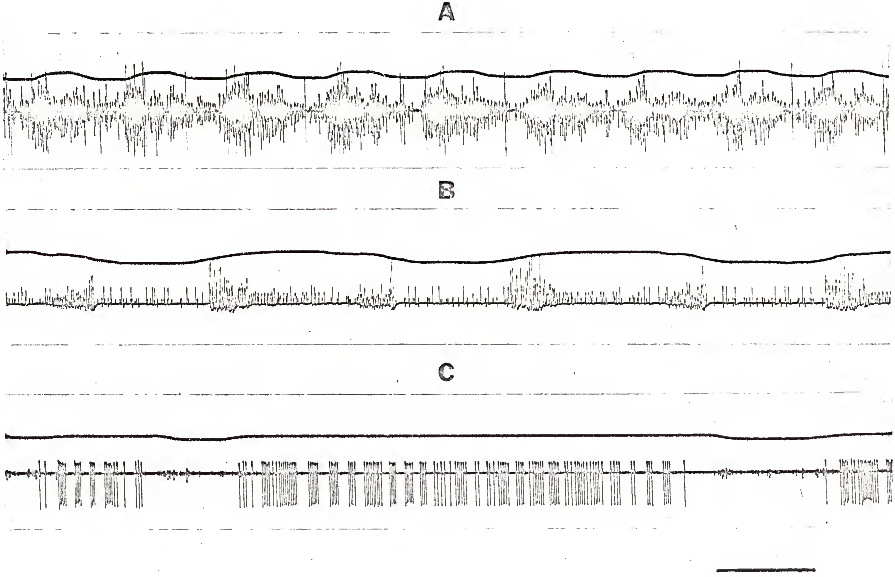
Rotation-sensitive units in the central nervous system were believed to be of a high order primarily because of the considerable lability of their response. Whereas first-order units from the statocyst located in the central component of the antennulary nerve were often active for several hours, higher-order units in the central

Figure 14. A.- Afferent activity in the central component of the antennulary nerve. Deflection of the upper trace indicates rotation of the statocyst.

B.- Rotation units in a small bundle of the central component. Deflection of the upper beam indicates rotation of the statocyst.

C.- A single position unit in the central component. The upper beam indicates that the statocyst has been rotated ventrally several degrees and held.

Time mark: .5 sec.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nervous system were seldom active for more than twenty minutes. The gradual decline in activity observed in these units after twenty minutes was attributed to transmission failure at synaptic sites in the antennulary neuropile resulting from reduced oxygen levels at these sites. The inability of the circulatory system to supply sufficient quantities of oxygen was believed to be due to the considerable loss of blood which accompanied ventral exposure of the cerebral ganglion.

In addition to lability of response, higher-order units differed from first-order units in frequently being more repetitive (compare Figure 14A with Figure 16A and B). Finally, stimulation of the central component of the antennulary nerve produced activity in both the heterolateral circumesophageal connective and the homolateral optic nerve. Figure 15A shows a single unit in the heterolateral connective driven by electrical stimulation of whole bundles in the central component of the antennulary nerve. Figure 15B shows repetitive firing in a single unit in the homolateral optic nerve in response to the stimulation of first-order units inside the statocyst near somata. Stimulation of the heterolateral connective and homolateral optic nerve did not evoke activity in the central component of the antennulary nerve.

Evoked Activity in the Circumesophageal Connectives and Thoracic Leg Nerves

Units sensitive to rotation of the statocysts were found more consistently in the connectives than anywhere else in the central nervous system. Before recording from the connectives, they were

Figure 15. A.- A single higher-order unit in one of the circumesophageal connectives responding to electrical stimulation of the homolateral central component of the antennulary nerve. Frame #1: subthreshold stimulus (1.5v). Frame #2: threshold stimulus (3v). Frame #3: suprathreshold stimulus (8v). Time mark: 10 msec.

B.- A repetitive higher-order unit in one of the optic nerves responding to electrical stimulation of receptor neurons at the base of the statocyst. Stimulus strength: 60v; stimulus duration: 6 msec. Time mark: 25 msec.

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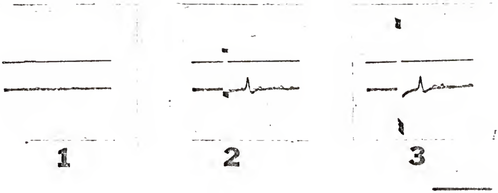

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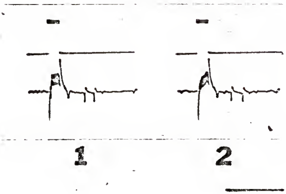
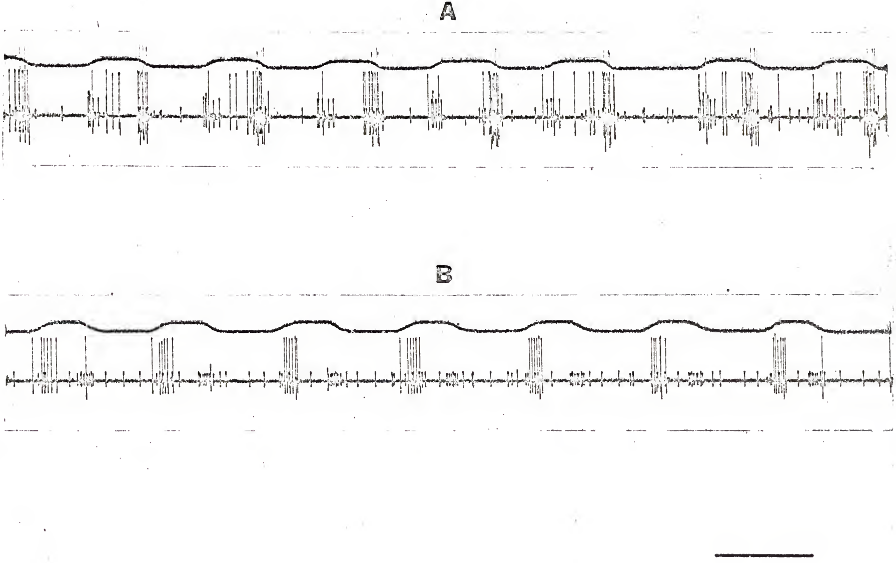


Figure 16. A.- Evoked activity in one of the circumesophageal connectives in response to rotation of the homolateral statocyst.

B.- Evoked activity in one of the connectives in response to rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In both A and B, an upward deflection of the upper trace indicated dorsal rotation of the statocyst.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sectioned two or more centimeters caudal to their point of entrance into the cerebral ganglion. The portion of the connective still attached to the ganglion was then lifted out of the body fluid with a hook electrode. The connectives were sectioned to insure that recorded activity was from descending units.

Figure 16A shows the responses of higher-order units in a connective during alternate dorsal and ventral rotation of the statocyst on the same side. Rotation in either direction was sufficient to evoke some activity, but the largest burst accompanied ventral rotation. Units in the homolateral connective exhibiting constant characteristics were studied in 35 preparations. The time course of the burst was determined by a single unit ($C_{hom.}$-1) which fired nine or ten times in the burst at an irregular rate. The burst duration was never constant and varied between 50 and 100 milliseconds for a 100-millisecond rotation. A second unit ($C_{hom.}$-2) in the burst fired intermittently three or four times during a burst, occasionally continuing after $C_{hom.}$-1 had finished. $C_{hom.}$-2 always exhibited a considerably greater spike amplitude than $C_{hom.}$-1 and fired with varying delay after the onset of $C_{hom.}$-1. There was no measurable delay between the rotational stimulus and the onset of the burst in the connective.

Evoked responses in the heterolateral connective during rotation of the statocyst were studied in 27 preparations. A single large unit ($C_{het.}$-1) fired repetitively at 100 spikes per second rate in response to dorsal rotation of the statocyst. The interspike interval was constant at ten milliseconds. A second repetitive unit in the heterolateral connective of distinctly smaller amplitude ($C_{het.}$-2) responded to ventral rotation of the statocyst at a rate approaching 125 spikes per second (Figure 16B). Repetitive firing in this unit varied from 60 to 100 milliseconds for a 100-millisecond rotational stimulus. There was no measureable latency between stimulus and response. Both homolateral and heterolateral responses were abolished when cell bodies in the region of the thread hair sensory cushion were destroyed.

As mentioned above, rotation of one of the statocysts produced stereotyped movements of the pereiopods. The extent of movement was graded in the five pairs of legs, with the greatest movement occurring in the fifth pair (modified in Callinectes for swimming) and the least occurring in the first pair. In a crab clamped ventral-side up, the response to dorsal rotation of a statocyst was most obvious in the heterolateral fifth leg. Movement in the homolateral fifth leg was less pronounced yet greater than movement in any of the other four pairs of walking legs. This relationship held for all other pairs of legs, with the heterolateral response always being greater than the homolateral one.

The phasic movements resulting from dorsal rotation of a statocyst are fundamentally the same in all five pairs of legs, despite the anatomical modifications for swimming in the fifth pair. In the first four pairs of legs the response is initiated by a flexion of the whole leg at the joint between ischiopodite and meropodite. This is followed by a more pronounced flexion of distal portions of the leg between meropodite and carpopodite. The distal two pereiopod segments (the propodite and dactylopodite) bend caudally with the dactylopodite extending slightly. The effect of these movements is to bring the legs in close to the ventral body wall.

Movements in the fifth pereiopods are basically the same as those just described in the other legs, although more pronounced. The one observable difference pertains to the direction of leg flexion. Whereas the anterior pairs of legs flex medially toward the body in a vertical plane, the fifth pair flex caudally in a more horizontal plane. The path described by the broadly flattened distal segments of the fifth legs is not a straight line but rather an arc which results from a slight ventral movement of the limb as it moves caudally. After the initial rapid phasic movement, all legs passively return to their original position.

As all leg movements were essentially the same, a careful analysis of units was undertaken in the heterolateral fifth pereiopods only, where the response was most pronounced. Figure 17A shows evoked responses in a fifth leg nerve exposed by removing the ventral body wall and associated musculature. The nerve was cut distally at its point of entrance into the leg and lifted above the fluid-air interface on a single electrode. A minimum of three units can be recognized in the bursts resulting from dorsal rotation of the heterolateral statocyst. A large amplitude nonrepetitive unit fires three times in Figure 17A, together with two more repetitive units of medium amplitude. The larger of the repetitive units fires several times in each burst with no consistent interval between spikes. The smaller unit fires sporadically three or four times in each burst. Figure 17B shows activity recorded from the distal portion of a fifth leg nerve inside the leg itself. Here only two consistent units were found. Figure 17C displays activity in a heterolateral fourth leg nerve sectioned distally and recorded from inside the body cavity. On this one occasion, a single, spontaneously active unit was observed to increase in frequency from 30 spikes per second to 70 spikes per second in response to ventral rotation of the heterolateral statocyst. Similar results were often obtained when recording from distal fifth leg nerves. A clear 40-to 50-millisecond latency between stimulus and response was noted in all recordings from the pereiopods. Active units in the heterolateral fifth pereiopods were studied in five preparations.

Evoked Activity in the Optic Nerves

Evoked responses to rotation of a statocyst were obtained in 40 preparations of the homolateral optic nerve and in 16 preparations of the heterolateral optic nerve. After peripheral sectioning of the homolateral nerve, two units were identified which responded repetitively to dorsal rotation and one which responded to ventral rotation. The two units responding to dorsal rotation had no consistent interspike interval and the number of spikes in a given burst varied

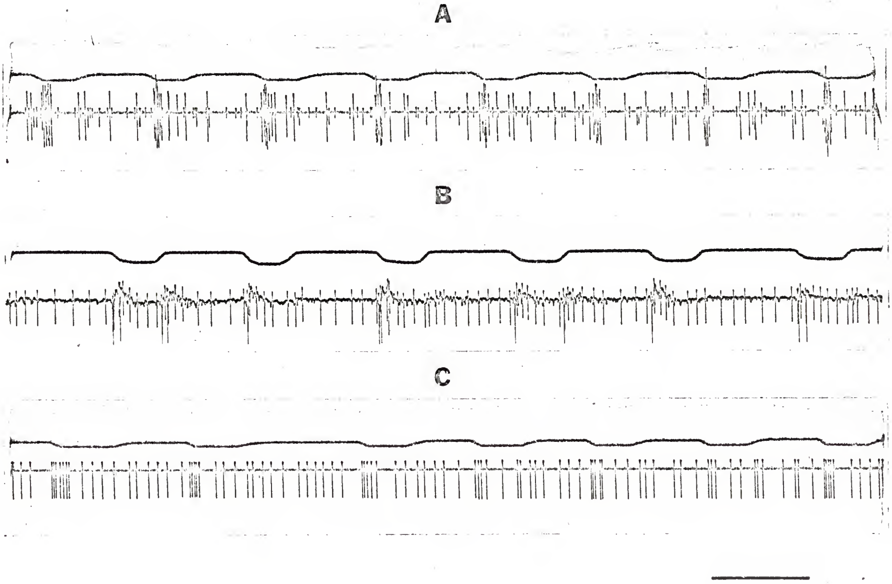
Figure 17. A.- Evoked activity in the proximal portion of one of the fifth pereiopods in response to rotation of the heterolateral statocyst.

B.- Evoked activity in the distal portion of one of the fifth pereiopods in response to rotation of the heterolateral statocyst.

C.- Evoked activity in the proximal portion of one of the fourth pereiopods in response to rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In A, B and C, a downward deflection of the upper trace indicates dorsal rotation of the statocyst.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B
C

considerably. Neither unit fired at a fixed interval following the onset of other, although the unit with the smaller amplitude usually followed the other (Figure 18A). The delay in onset of the second unit caused great differences in burst duration between preparations, but burst durations were reasonably constant in a given preparation. The single unit responding to ventral rotation generally fired several times very rapidly and was quite consistent (Figure 18A). Evoked responses were complicated by spontaneous efferent activity in the nerve and by units responding in a nonrepetitive fashion to rotation of a statocyst.

Responses to rotation in peripherally sectioned heterolateral optic nerves are represented in Figure 18B. These responses did not show any pattern and were not analyzed. No clear latency of response could be established in either the homolateral or the heterolateral optic nerve. Again, destruction of cell bodies at the base of the thread hair sensory cushion prevented further evoked activity in these nerves.

Evoked Activity in the Oculomotor Nerves

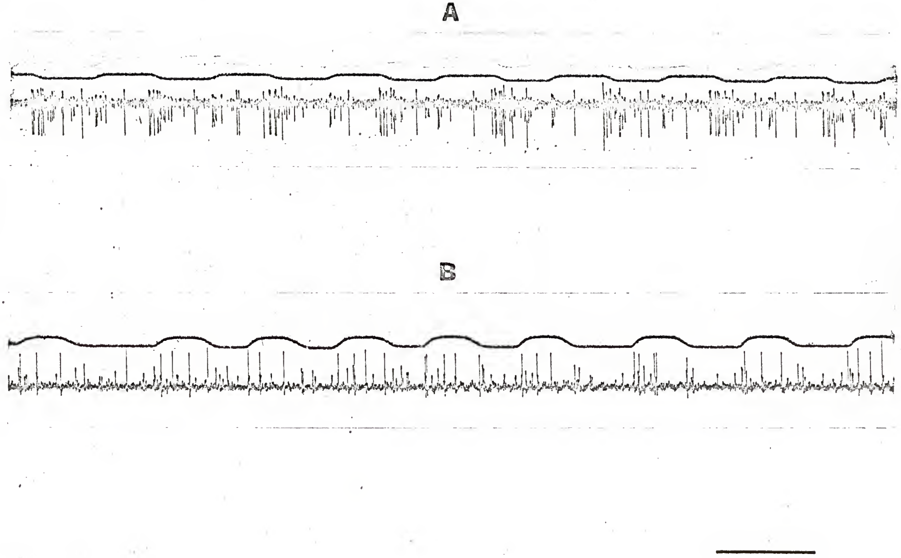
Evoked responses in the oculomotor nerves (studied in 32 preparations) were among the most consistent found anywhere despite the fact that no active unit fired more than two or three times in a burst. Rotation of one statocyst produced activity in both oculomotor nerves, with the greatest response occurring in the homolateral nerve. The response in this nerve was largest with ventral rotation of the statocyst, although dorsal rotation frequently

Figure 18. A.- Evoked activity in one of the optic nerves in response to rotation of the homolateral statocyst.

B.- Activity in one of the optic nerves during rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In both A and B, a downward deflection of the upper trace indicated dorsal rotation of the statocyst.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evoked some activity (Figure 19A). The duration of the burst produced by ventral rotation was between 40 and 50 milliseconds and there was no apparent response latency. Despite the fact that as many as eight or more units frequently were active during a burst and none were particularly repetitive, the form of the burst was remarkably consistent from preparation to preparation.

The time course of each burst was determined by small amplitude units, with elements of larger amplitude active midway through the burst. No clear response pattern emerged from the analysis of activity produced by dorsal rotation in the homolateral and heterolateral nerves and no activity at all was recorded in the heterolateral nerve when the statocyst was rotated ventrally. All evoked activity ceased after the destruction of the innervation of the thread hairs. The rotation of statocysts in intact crabs did not result in eyecup movements.

Evoked Activity in the Antennal Nerves

Evoked responses in the antennal nerve were obtained 50 percent of the time. Even when present, responses were inconsistent and consecutive rotation of the statocyst failed to excite the same units each time. Ventral rotation was the most effective stimulus when activity was found in either nerve (Figure 20A and B). In intact animals, ventral rotation was sometimes accompanied by lateral twitching of the homolateral antenna.

Figure 19. A.- Evoked activity in one of the oculomotor nerves in response to rotation of the homolateral statocyst.

B.- Evoked activity in one of the oculomotor nerves in response to rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In both A and B, an upward deflection of the upper trace indicates ventral rotation of the statocyst.

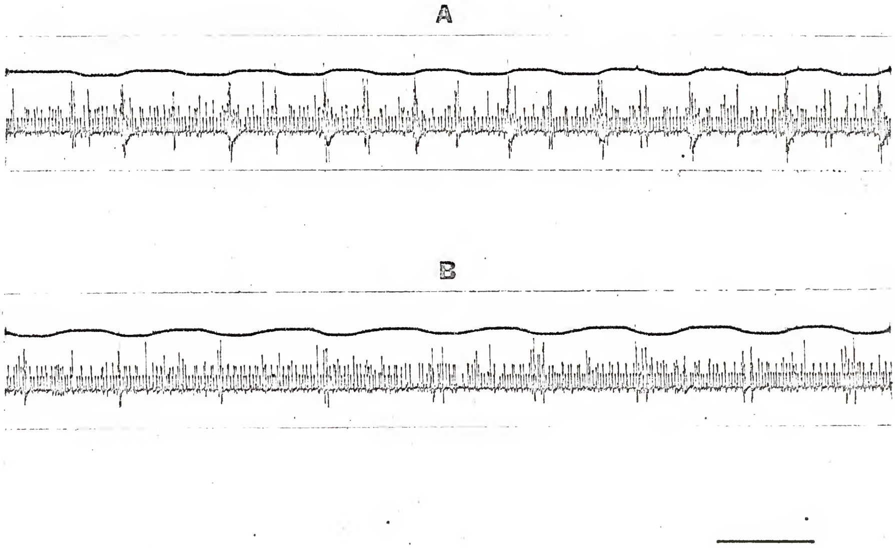
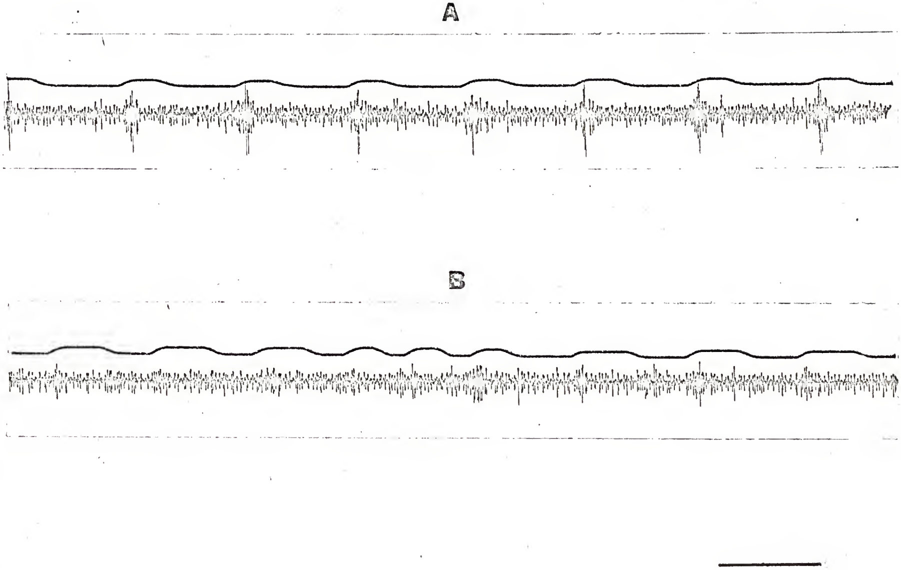

A
B

Figure 20. A.- Evoked activity in one of the antennal nerves in response to rotation of the homolateral statocyst.

B.- Activity in one of the antennal nerves during rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In both A and B, an upward deflection of the upper trace indicates ventral rotation of the statocyst.


A
B

Evoked Activity in the Medial Component of the Antennulary Nerve

Evoked responses in peripherally sectioned medial components were consistent in 11 preparations. These responses were characterized by a single, large amplitude unit (M.C.$_{\text{hom.}}$-1) firing repetitively in response to both dorsal and ventral rotation of the homolateral statocyst (Figure 21A). The same unit or a similar one (found in two preparation) fired with fewer spikes in a burst to dorsal and ventral rotation of the contralateral statocyst (Figure 21B). M.C.$_{\text{hom.}}$-1 is unique in that it occasionally responded continuously when the homolateral statocyst was rotated ventrally and held (Figure 21C). Activity in this unit was delayed about 70 milliseconds following either homolateral or heterolateral rotation. Neither interspike interval nor response duration was constant in this unit.

Evoked Activity in the Lateral Component of the Antennulary Nerve

Evoked activity in peripherally sectioned lateral components was investigated in 29 preparations. Repetitive units were present which responded to either dorsal or ventral rotation of the homolateral statocyst. One unit of considerable amplitude (L.C.$_{\text{hom.}}$-1) fired at a constant rate of 180 spikes per second (5-millisecond interspike interval) to dorsal rotation of the homolateral statocyst. A second unit (L.C.$_{\text{hom.}}$-1) of smaller amplitude fired repetitively but irregularly to ventral rotation of the homolateral statocyst (Figure 22A). A number of nonrepetitive units frequently accompanied both dorsal

Figure 21. A.- Evoked activity in the medial component of the antennulary nerve in response to rotation of the homolateral statocyst.

B.- Tonic response of a single unit in the medial component.

C.- Evoked activity in the medial component in response to rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In A, B and C, an upward deflection of the upper trace indicates ventral rotation of the statocyst.

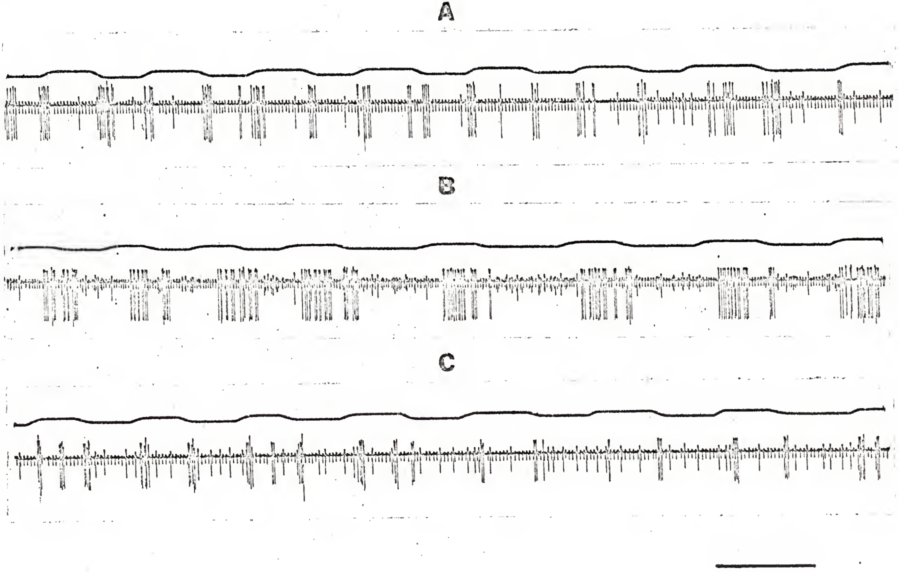
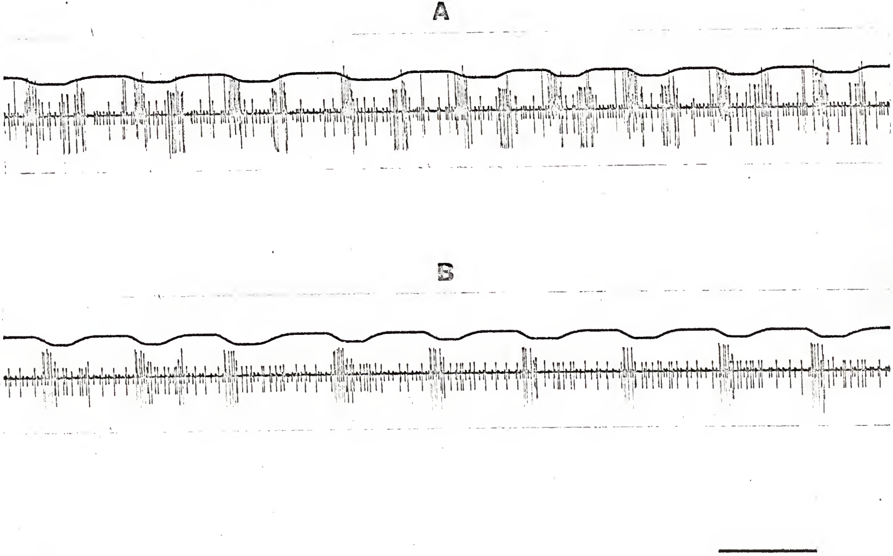

A
B
C

Figure 22. A.- Evoked activity in the lateral component of the antennulary nerve in response to rotation of the homolateral statocyst.

B.- Evoked activity in the lateral component in response to rotation of the heterolateral statocyst.

Time mark: .5 sec.

Note: In both A and B, an upward deflection of the upper trace indicates ventral rotation of the statocyst.


A
B

and ventral rotations. Both repetitive units were active for the duration of the 100-millisecond stimulus. A single large amplitude unit (perhaps L.C.$_{\text{hom.}}$-1) was activated by dorsal rotation of the heterolateral statocyst. This unit fired at a fairly regular rate approaching 100 spikes per second. Despite the difference in rate, this unit may yet be L.C.$_{\text{hom.}}$-1 as it was of approximately the same amplitude and was always active in preparations containing L.C.$_{\text{hom.}}$-1. This unit fired for 50 milliseconds in response to a 100-millisecond rotation. Both homolateral and heterolateral responses were delayed 20-30 milliseconds.

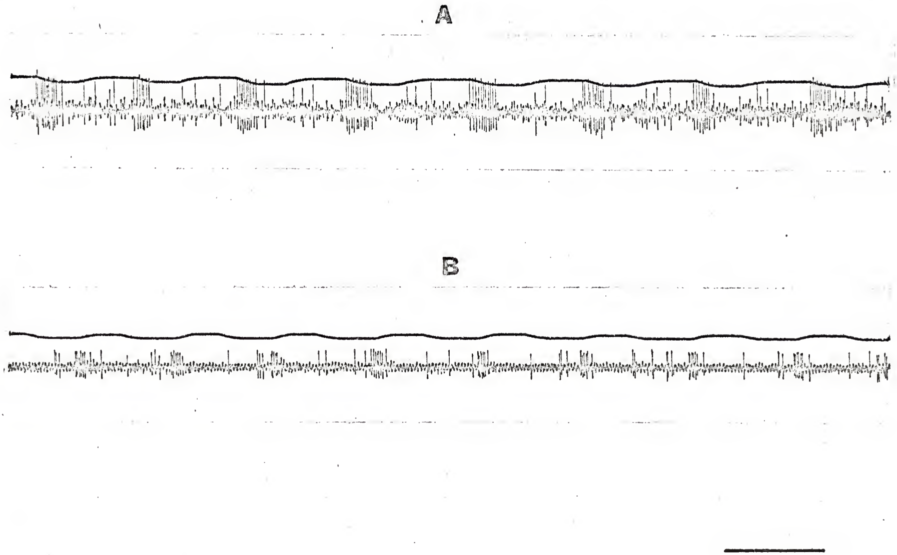
Evoked Activity in the Antennulary Neuropile

Attempts made to record extracellular activity in the antennulary neuropile were not fruitful. However, two units were identified in seven preparations. Both were located deep in dorsal regions of the neuropile. One unit responsive to dorsal rotation of the homolateral statocyst (N$_{\text{hom.}}$-1) was found in the caudo-medial portion of the neuropile. A second unit (N$_{\text{hom.}}$-2), sensitive to ventral rotation, was located in the caudo-lateral portion. N$_{\text{hom.}}$-1 fired at a rate of 80 spikes per second for the duration of the rotational stimulus, which approached 140 milliseconds (Figure 23A). In contrast, N$_{\text{hom.}}$-2 fired at 115 spikes per second rate for 60 milliseconds in response to a 100-millisecond stimulus (Figure 23B). The latency from stimulus to response in these units was too brief to be resolved with the recording technique used.

Figure 23. A.- Repetitive unit in the antennulary neuropile responding to dorsal rotation of the homolateral statocyst.

B.- Repetitive unit in the antennulary neuropile responding to ventral rotation of the homolateral statocyst.

Time mark: .5 sec.


A
B

Spontaneous Discharges in the Optic and Oculomotor Nerves Responsible for Eyecup Withdrawal

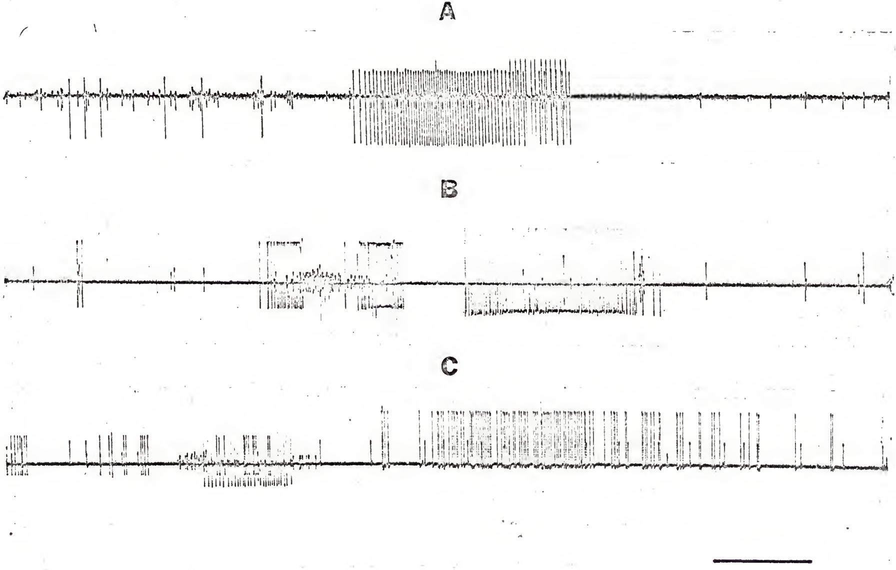
Burrows (1967), Burrows and Horridge (1968) and Sandeman (1967, 1969a, 1969b) have described in some detail the nature of efferent discharges in the optic and oculomotor nerves of *Carcinus* responsible for eyecup withdrawal. In *Callinectes* the spontaneous activity responsible for the withdrawal response appears to be similar to that which occurs in *Carcinus*. Figure 24A and Figures 24B and C show spontaneously occurring bursts of activity in peripherally sectioned optic and oculomotor nerves, respectively. These bursts of activity accompany withdrawal of the eyecup on the opposite side of the crab, where the optic and oculomotor nerves are still intact. The simultaneous withdrawal of both eyecups is frequently observed in whole animals, although the individual withdrawal of each eyecup is common. It is suggested that activity recorded in the sectioned nerves is the same as that occurring in the heterolateral nerves and therefore responsible for eyecup withdrawal.

The spontaneous discharge in the optic nerve was rather labile and only rarely observed, yet was easily studied as it was composed of only two repetitive units. Each burst was initiated by a unit which fired at a rate of 50 spikes per second, increased to 100 spikes per second midway through the burst and then returned to a 50-spike per second rate before stopping. A second unit entered the burst roughly three-quarters of the way through and fired at a constant rate of 40 spikes per second. Both units stopped firing at roughly the same time.

Figure 24. A.- Eyecup withdrawal discharge in a fresh preparation of one of the optic nerves. Time mark: .5 sec.

B.- Eyecup withdrawal response in a fresh preparation of one of the oculomotor nerves. Time mark: 1 sec.

C.- Eyecup withdrawal in a deteriorating preparation of one of the oculomotor nerves. Sporadic asynchronous firing of the first unit in the burst is apparent. Time mark: 1 sec.


A
B
C

The duration of the burst (frequently dependent upon the length of time the first unit fires) varied considerably from preparation to preparation. After long periods of recording, interspike intervals increased and fewer spikes were generated in a burst.

Spontaneous activity in one of the oculomotor nerves involves four units. Each burst was initiated by a unit of considerable amplitude which fired repetitively until reaching a frequency of 80 spikes per second, at which time it stopped abruptly. Just prior to or immediately after this unit stopped, two or more small amplitude units began firing repetitively. These units exhibited no observable pattern of firing. A single large element, firing at a consistent rate of 140 spikes per second, began while both small units were still active. After both small amplitude units stopped and while the largest unit was still firing, the unit initiating the burst fired again at a frequency approaching 140 spikes per second. This unit terminated the burst after the largest unit stopped. The length of time each unit fired in the course of a burst varied from preparation to preparation, but the order in which units entered the burst did not change (compare Figure 24B with Figure 24C). This discharge pattern was observed more than ten times in each of six different preparations.

Effect of Spontaneous Activity on Evoked Responses in the Homolateral Oculomotor Nerve and the Lateral Antennulary Nerve

In view of the occasional spontaneous repetitive activity in certain efferent ganglionic nerves (specifically the oculomotor and lateral antennulary nerves), an attempt was made to determine whether or not this activity had any observable effect on evoked responses in these nerves. Results are shown in Figure 25. In Figure 25A, the withdrawal discharge does not inhibit evoked activity in the homolateral oculomotor nerve despite the fact that most background activity in the nerve is inhibited before, during and after the discharge. Figures25B and C show the effect of a single repetitive unit on evoked responses in the lateral antennulary nerves. In the homolateral nerve the spontaneous unit partially inhibits the repetitive response of L.B.$_{\text{hom.}}$-1. In the heterolateral nerve, the unit responding to dorsal rotation of the statocyst is totally inhibited. The failure of spontaneous activity in the oculomotor nerve to inhibit evoked responses in this nerve was observed in four preparations. The inhibitory effect of the spontaneously active unit in both lateral antennulary nerves was observed in three preparations.

Discussion

A Comparison of Primary Afferent Input from the Statocyst in Callinectes and Homarus

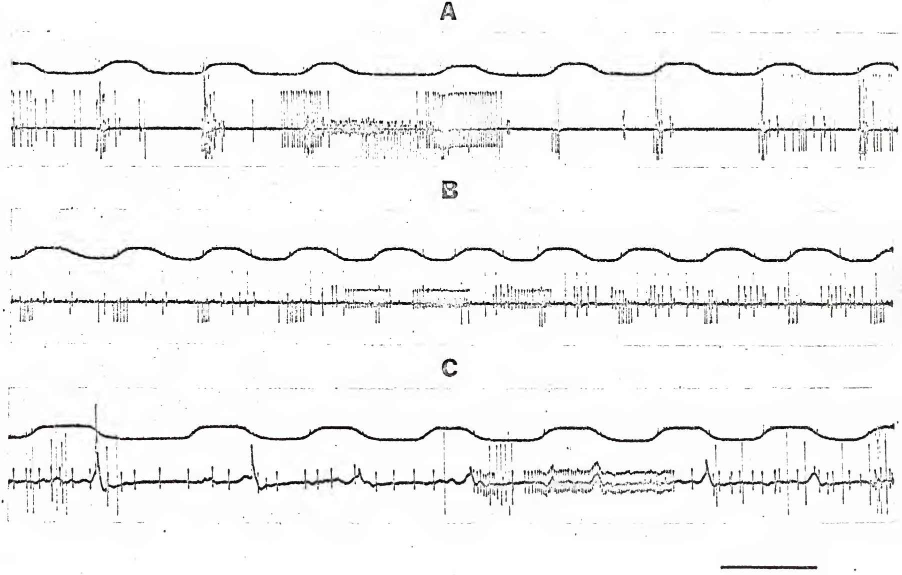
Two fundamental types of statocyst receptors have been described in Homarus: position receptors, which exhibit a tonic response in the form of a non-adapting repetitive discharge to maintained positions of the experimental animal as rotated about its axes, and rotation receptors, which respond phasically to movement only (Cohen, 1955, 1960). Significantly, both of these receptor types were observed in Callinectes, but

Figure 25. A.- Failure of spontaneous activity in one of the oculomotor nerves to inhibit activity evoked in this nerve as a result of the rotation of the homolateral statocyst. An upward deflection of the upper trace indicates ventral rotation of the statocyst.

B.- Partial inhibition of evoked activity in unit $L.C._{hom.}$-1 during a burst of spontaneous activity in a unit in the lateral component of the antennulary nerve.

C.- Total inhibition of an evoked response in the lateral component on the side opposite the statocyst being rotated.

Time mark: .5 sec.


A
B
C

tonically responding position receptors were found less frequently than phasic rotation receptors. This fact is attributed to those mechanical limiting factors in the preparation which preclude rotation of either statocyst more than a few degrees. It is possible that considerably more position receptors could have been demonstrated if a greater arc of rotation had been possible.

In macruran decapods, the mass of the statolith under the influence of gravity produces a bending of the statolith hairs. When an animal is rotated, certain hairs are bent to a greater extent than others by the weight of the statolith. Holding an animal in one position causes some hairs to remain bent, producing the tonic response characteristic of position receptors. Until recently (Schöne and Steinbrecht, 1968) each statolith hair was believed to be innervated by only one sensory neuron (Cohen, 1960), and it seemed likely that the only response statolith hairs were capable of was a tonic one.

The study of Schöne and Steinbrecht (1968) indicates that the statolith hairs, in some cases at least, are triply innervated. Electrophysiological confirmation of this has come from Patton (1969), who describes activity in three distinct sensory neurons produced by the mechanical displacement of a single statolith hair (in Homarus). The output of each neuron is different, with one responding phasically, another tonically and the last a tonic discharge at rest which falls to zero when the hair is moved laterally. It is therefore not possible to assign to the statolith hairs one specific functional role, although these receptors are clearly the ones best adapted for delivering tonic inputs to the central nervous system.

In Homarus, the rapidly adapting (i.e., phasic) responses of rotation receptors have been linked to the behavior of the thread hairs under the influence of fluid movements in the lumen of the statocyst (Cohen, 1955, 1960). In this study, the destruction of cell bodies in the region of the thread hair sensory cushion stopped phasic activity in sensory bundles from the statocyst, as well as in specific higher-order neurons in the central nervous system of Caliinectes. In both of these forms, the thread hair receptors clearly effect phasic outputs. In addition, since these receptors are sensitive to fluid movements and are not affected by the mass of the statolith (in Homarus), it is difficult to conceive of their initiating any form of tonic output.

One interesting aspect of tonic and phasic input from the statocyst in Homarus and Callinectes is that both of these inputs have a directional quality. Figure 14B shows phasic activity in first-order units from thread hairs in the statocyst of Callinectes. It is readily apparent that units responding to rotation in one direction are different from those responding to movement in the opposite direction. The tonic response of the unit in Figure 14C is also specific with regard to direction. In Homarus, the mechanical displacement of thread hairs in one direction evokes a burst of activity, followed by a depression of activity below the resting discharge, followed by a return to the resting discharge. Displacement in the opposite direction has the opposite effect: depression, burst, resting discharge (Cohen, 1956).

The study of Schöne and Steinbrecht (1969) concerning the fine structure of the statolith hairs of Astacus fluviatilis appears to explain these findings. In Astacus, the base of each statolith hair is hinged on one side. Each hair is innervated by three sensory neurons whose dendrites merge into a chitinous cord which enters the hair and attaches to the shaft opposite the hinge. Movement of the shaft in the direction of the hinge stretches the cord, which in turn could cause depolarization of the dendritic endings of the receptors, producing a repetitive discharge. Movement of the shaft in the opposite direction seems to minimize stretching of the cord and could produce the response depression observed in Homarus by Cohen (1956). In many respects the overall structure of this system resembles that of chordotonal proprioceptors in the legs of Carcinus (Whitear, 1962).

Two possible explanations are offered for the problem of why rotation of a statocyst in one direction activates phasic receptors apparently not activated by rotation in the opposite direction. It may be that bending of the entire row in one direction stimulates some members of the row and inhibits others, with bending in the opposite direction having the reciprocal effect. This can be explained on the basis of Schöne's work by assuming that the hinged bases of the hairs do not all occur on the same side of the thread hair row.

A second possibility is that rotation in either direction sets up fluid movements inside the sac which flow over the row in the same direction. These movements may be qualitatively different enough to result in differential stimulation of the receptor hairs. Further research is necessary to determine whether or not either of these explanations is correct.

Evidence Supporting a Conclusion that Evoked Activity in the Central Nervous System Produced by Rotation of the Statocysts is of a High Order

Over the course of each experiment, it has been found that evoked activity declines in first-order sensory elements from the statocyst. A reasonable explanation for this is transmission failure at the synapse resulting from reduced oxygen levels in the antennulary neuropile. The decreased efficiency of the circulatory system is attributed to the opening of the haemocoel and the slow but steady loss of blood which follows. Exposure of the haemocoel is always accompanied by a gradual decline in vigor in these crabs.

Additional evidence supporting the statement that one or more synapses are interposed between primary afferent elements from the statocyst and units in the central nervous system comes from experiments in which primary afferent elements from the statocyst were stimulated electrically. Electrical stimulation of sensory bundles from the statocyst was sufficient to drive units in the homolateral optic nerve and heterolateral circumesophageal connective. In contrast, antidromic stimulation of these nerves evoked no response in afferent units from the statocyst. These results suggest that the synapses involved are rectified and that synaptic transmission is chemically mediated.

Units presumed to be of a high order were characteristically of greater amplitude and were often more repetitive than first-order units from the statocyst. In support of this, Wiersma and Mill (1965) have observed the primary afferent units descending in the circumesophageal connectives of Procambarus clarkii tend to be small in size. Activity in small units such as these would probably not be monitored in experiments in which whole nerves were recorded from, as is the case in this study. These facts support the conclusion that evoked activity in the central nervous system is of a higher order.

Comments on Higher-Order Neurons Sensitive to Rotation of the Statocysts

Figures 16 through 22 make it clear that the bursts of activity evoked by rotation of the statocysts in the various efferent ganglionic nerves are consistent in a given preparation. With the exception of the antennal nerve, burst patterning in each nerve was consistent with that found in the same nerve in different preparations. Higher-order neurons activated by rotation of the statocysts were classified as nonrepetitive, regularly repetitive and irregularly repetitive. Nonrepetitive units were found consistently in many preparations and will not be considered here. Regularly repetitive and irregularly repetitive units found consistently in many preparations are listed in Table 1.

TABLE 1

REPETITIVE UNITS IN THE CENTRAL NERVOUS SYSTEM RESPONDING TO ROTATION OF THE STATOCYSTS

Unit	Form of Response	Rotational Sensitivity		Times Found	Relative Size	Estimated Rate of Response (Spikes/Second) (Regularly Repetitive Units Only)	Response Latency
		Dorsal	Ventral				
Circumesophageal Connectives							
$C_{hom.}$-1	Irregularly Repetitive		X	35+	Large		None Apparent
$C_{het.}$-1	Regularly Repetitive	X		27+	Large	100	None Apparent
$C_{het.}$-2	Regularly Repetitive		X	27+	Small - Medium	125	None Apparent
Antennulary Nerve Medial Component							
M.C.$_{hom.}$-1	Irregularly Repetitive	X	X	11	Large		Up to 70 msec
Lateral Component							
L.C.$_{hom.}$-1	Regularly Repetitive	X		29+	Large	180	Up to 30 msec
L.C.$_{hom.}$-2	Irregularly		X	29+	Large		None Apparent
Antennulary Neuropile							
$N_{hom.}$-1	Regularly Repetitive	X		7	Large	80	None Apparent
$N_{hom.}$-1	Regularly Repetitive		X	7	Large	115	None Apparent

The three units descending in the circumesophageal connectives are believed to be interneurons, as they arise in the cerebral ganglion and synapse in the thoracic ganglia. $C_{het.}$-1 is clearly interneuronal, driving motoneurons in the heterolateral thoracic pereiopods responsible for stereotyped movements of these thoracic appendages. Units found in the antennulary neuropile ($N_{hom.}$-1 and $N_{hom.}$-2) would appear to be interneurons restricted to the cerebral ganglion, as their rates of response do not correspond to those of units monitored in efferent ganglionic nerves (Table 1).

In addition to motoneurons passing to muscles of the eyecup (Horridge and Sandeman, 1964), the optic nerves carry efferent mechanoreceptive interneurons to the optic ganglia (Bush, Wiersma and Waterman, 1964). The results of this study have shown that certain elements coursing peripherally in these nerves respond to rotation of the statocysts. However, activity in these units was not associated with eyecup movements and the classification of these units as motoneurons or interneurons was not possible.

Cohen (1956) and Patton (1969) have shown that the deflection of statolith hairs in Homarus activates elements in the heterolateral oculomotor nerve which cause a depression in the rate of discharge of muscle 23B in the eyecup (Cochran, 1935). These findings suggest that the oculomotor nerves contain inhibitory elements capable of being activated by input from the statocysts. In Callinectes, certain elements in the oculomotor nerves are influenced by input from the thread hair receptors (shown by the fact ablation of these receptors leads to a cessation of evoked activity in these nerves). It is noteworthy that in Callinectes activity in the oculomotor nerves does not result in eyecup movement, indicating that these elements may be inhibitory. However, the possibility that some units are motoneurons cannot be dismissed.

Active units in the medial components of the antennulary nerves (passing to muscles of the antennulae) and lateral components of the antennulary nerves (passing to muscles of the basal segments) are either motoneurons or inhibitory elements. Rotation of the basal segments and antennulae prevented the observation of compensatory movements by these structures, and consequently the identification of motoneurons was not possible.

The single repetitive unit ($M.C._{hom.}$-1) in the medial component was the only unit found which responded both tonically and phasically to input from the statocyst. The tonic discharge suggests that this element receives input from the hook hair receptors in addition to input from the phasically responding thread hairs. This element may establish the position of the antennule during and after changes in posture, either actively by muscle excitation or passively through peripheral inhibition. Presumably a tonic discharge results in a stable, maintained position of the antennule.

In considering the form of the repetitive outputs of units listed in Table 1, the findings of Kennedy and Preston (1960), Kennedy and Mellon (1964), Takeda and Kennedy (1965) and Maynard (1967) concerning the decapods Procambarus clarkii and Panulirus argus are particularly relevant. It was mentioned earlier that interneuronal elements found in the antennulary neuropile of decapods possess widespread dendritic arborizations and appear capable of intracellular communication by means of propagated spikes (Maynard, 1967). Similar capabilities are attributed to the branches of higher-order units (interneurons and motoneurons) by Takeda and Kennedy (1965) who state:

> In many if not all these crayfish interneurons, depolarizations produced by the branch spikes summate temporally and spatially at an impulse-generating site on the main axon which determines the output discharge of the neuron; the mode of summation is determined by the geometry of the branches involved. . . . Several branch spikes may converge to evoke a single axon spike, while temporally asynchronous ones may cause repetitive discharges of the main axon by bringing the level of depolarization at the spike-generating site to a critical value. . . . The pattern of such discharges may be rather irregular when the contribution of branch spikes is discrete, since it will depend upon the arrival time of the latter. However, firing may be regular when many branch spikes sum to cause a long lasting, smooth depolarization of the spike-generating site on the main axon.

Sandeman (1969a, 1969b) has shown that the impulse-generating site of a single motoneuron in the optic nerve of Carcinus is separated from certain synaptic inputs to the neuron by roughly 300μm of membrane. In this respect his findings correspond to those of Maynard (1967) and others. However, Sandeman states that the membrane between impulse-generating site and synaptic inputs is incapable of actively propagating spikes and therefore differs from comparable regions of similar neurons in Procambarus and Panulirus. In place of all-or-none activity, smooth, graded depolarizations capable of temporal and spatial summation occur in this region.

The results of this study appear particularly meaningful in view of these recent discoveries. The outputs of the higher-order neurons described here may be explained on the basis of the temporally asynchronous input to the antennulary neuropile from the statocyst receptors and the anatomical patterning of this input of the dendritic branches of these neurons. Post-synaptic events in these central neurons (whether graded or propagated) sum temporally and spatially to produce the regularly and irregularly repetitive outputs characteristic of these elements. Figure 14A makes it clear that phasic input from the statocyst is asynchronous, and Table 1 shows that the responses of consistent higher-order units are either regularly or irregularly repetitive.

The inhibitory effect of spontaneous activity on evoked responses in certain of the efferent ganglionic nerves can be explained on the basis of Maynard's (1967) discovery that separate spike-initiating sites may exist in higher-order elements characteristic of diffuse neuropile in the cerebral ganglion of Panulirus. Inputs at certain strategic loci could then make other inputs ineffective through intracellular occlusion, thereby exerting an inhibitory effect.

The Functional Significance of Pereiopod Movements Resulting from Dorsal Rotation of the Statocysts

In crabs clamped ventral-side up, rotation of a single statocyst dorsally produces characteristic movements of the thoracic pereiopods which are most pronounced in the heterolateral fifth appendage. Movements in the heterolateral appendages generally are believed to result from repetitive activity in a single large interneuron ($C_{het.}$-1) descending in the heterolateral circumesophageal connective (Figure 16B). This unit appears responsible for bursts of activity in three motoneurons in the heterolateral fifth leg nerve and at least one motoneuron in the heterolateral fourth leg nerve (Figure 17B and C).

The response in the heterolateral fifth pereiopod, while considerably greater than the response in any of the other legs, is still little more than an intention movement. It is apparent, however, that the response is a miniaturized version of the "sculling" movements which are characteristic of this broad paddle-like appendage.

In any attempt to relate these movements to meaningful behavior, the problem of unilateral rotation of the statocyst must be considered. It cannot be inferred that rotation of one statocyst signals rotation of the whole animal about the transverse axis. In this case the central nervous system would receive comparable information from both statocysts and initiate synchronized sculling in both of the fifth periopods. A more reasonable explanation is that unilateral rotation signals a lateral or antero-lateral rotation of the whole animal about the longitudinal axis. Rotation in this plane produces a downward movement of the statocyst on the lead side and an upward movement of the statocyst on the trailing side. For an animal in an inverted position, this rotation is directed toward a return to the animal's normal dorsal-side up posture. The coupling of this side-over-side rotation with the normal swimming movements of the heterolateral fifth periopod accelerates the return to proper orientation with respect to gravity. Side-over-side movements, with associated asynchronous "sculling" of the fifth pereiopods, are frequently observed in free-swimming crabs.

The phasic limb movements described result from brief, often repetitive bursts of activity in sensory, interneuronal and motoneuronal elements originating in the central nervous system of Callinectes. These movements appear meaningful in terms of orienting the animal in the course of changes in position. Callinectes is a swimming crab and therefore subject to movements in three planes, while many other decapods are generally limited to movements in the horizontal plane only. It is clearly adaptive for Callinectes to be capable of reorientation while swimming. Stimuli initiating rapid (and limited) whole-body rotation would produce fluid movements inside the statocysts which would activate the delicate thread hairs. Hook (statolith) hairs might also be activated, but in view of the brief time course of these types of phasic stimuli and the subsequent rapid reorienting responses, it is difficult to postulate an important role for these receptors. It is more likely that in Callinectes the hook hairs are primarily responsible for long-lasting, tonic changes in posture.

Rotation of statocysts individually (and presumably rotation of the whole animal as well) activates primary afferent units from the statocyst. These units in turn excite higher-order elements in the antennulary neuropile. Repetitive activity was also observed in higher-order neurons leaving the central nervous system. These elements are believed to originate in the antennulary neuropile or in a synaptic field receiving input from this neuropile.

The repetitive activity in higher-order neurons responsive to phasic excitation of the statocysts has been linked to orienting behavior in Callinectes. It is suggested that the output of these neurons is the result of temporally asynchronous input from the statocyst impinging upon the dendritic branches of these elements. These neurons sum the resulting post-synaptic events (in the form of either graded depolarization or propagated spikes) at the impulse-initiating site of the main axon. The form of the repetitive output of the spike-initiating site is ultimately dependent upon the number and location of sensory endings on the dendritic branches and the location of these branches themselves with respect to the spike-initiating site.

The stereotyped activity produced in all of the efferent nerves to the appendages (including the eyestalks and antennules) by brief rotation of a single statocyst indicates that input from the statocyst is channeled into numerous and specific output lines within the antennulary neuropile. It has been shown that evoked activity in some of the thoracic leg nerves leads to pereiopod movements which precipitate the return to a dorsal-side up posture in Callinectes.

Despite the fact that evoked activity in the optic, oculomotor and antennulary nerves does not lead to obvious motor activity in the eyecups and antennules, the orientation of these appendages may nevertheless be affected by these discharges. Burrows and Horridge (1968) have shown that static compensatory responses in Carcinus result from changes in tonic activity in motoneurons passing to the muscles of the eyecups. It seems reasonable to infer that phasic input from one statocyst, perhaps coupled with other inputs, may result in motoneuronal activity responsible for phasic compensatory movements of these appendages.

As a final consideration, the works of Ewald (1892), Bethe (1897) and Lowenstein and Sand (1940) indicate that certain sense organs including the statocysts exert a continuous influence on central excitability levels. Cohen (1965) has shown that the function of the myochordotonal organs in Cancer magister is to set the excitability levels of muscles operating the leg joints in which they are located. The receptor muscles of the myochordotonal organs themselves receive specific motor fibers. As a consequence, changing the frequency of tonic activity in efferent axons to the receptor muscles can result in a change in the excitability levels of the main muscles of the joint. It is therefore possible that in Callinectes brief phasic input from the statocyst results in an increase in the general excitability level of the central nervous system by acting on the myochordotonal organs of the legs. The positive feedback of these receptors upon motoneurons of the legs then produces the movements observed.

GENERAL SUMMARY

Part I

1. Morphological features of the basal antennulary segments of Callinectes sapidus and the equilibrium organs (statocysts) contained within these segments are described.

2. The anatomy of receptor elements (hook, thread and group hairs) in the statocysts is presented. All three types are described for the first time in Callinectes and appear to be identical to comparable receptor hairs in the statocysts of Carcinus maenas, the European green crab.

3. Peripheral and central ramifications of neurons making up the three components of the antennulary nerve are described. Particular emphasis is placed on the distribution of the central component of the antennulary nerve which innervates the receptor hairs of the statocyst.

4. The gross anatomy of the central nervous system of Callinectes is described, particularly the region of the cerebral ganglion receiving sensory input from the statocyst: the antennulary neuropile.

Part II

1. Higher-order units in the central nervous system responding to a rapid, 3° rotation of one of the statocysts in the dorso-ventral plane are described.

2. All efferent nerves leaving the cerebral ganglion (the optic, oculomotor, antennulary and antennal nerves, together with the

circumesophageal connectives) are found to contain elements which respond to rotation of a single statocyst.

3. Activity in many of these units is repetitive, and all responses are restricted to the duration of the rotational stimulus (i.e., responses are phasic).

4. Evidence is presented indicating that the delicate thread hair receptors in the statocysts are the receptor type responding to fluid movements inside the statocysts resulting from rotation of the statocysts.

5. In addition to evoked activity in efferent nerves leaving the central nervous system, spontaneously occurring activity in these nerves is described. Of particular interest were bursts of activity in the optic and oculomotor nerves. These spontaneous discharges are believed responsible for the reflexive withdrawal of the eyecup in Callinectes.

6. Spontaneous repetitive activity of a single nerve in the lateral component of the antennulary nerve appeared to inhibit centrally activity evoked in the same nerve caused by rotation of the statocyst of the heterolateral side. Spontaneous activity in the oculomotor nerve had no effect on evoked activity in this nerve.

7. Dorsal rotation of a single statocyst frequently resulted in stereotyped movements of the heterolateral thoracic appendages, the pereiopods. These movements were particularly obvious in the fifth pereiopod, modified for swimming in Callinectes. It is suggested that a single, repetitive interneuron ($C_{het.}$-1) descending in the heterolateral

circumesophageal connective is responsible for movements observed in these appendages.

8. All of the findings listed above suggest that phasic input from the statocysts, acting on higher-order neurons in the antennulary neuropile, continually serves to adjust the position (attitude) of the various appendages.

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BIOGRAPHICAL SKETCH

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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